Enzyme and Microbial Technology 124 (2019) 17–22

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/enzmictec

Producing alcohol and salt stress tolerant strain of *Saccharomyces cerevisiae* by heterologous expression of *pprI* gene

S. Hossein Helalat[a,*], Shirin Bidaj[b], Sadaf Samani[c], Mohammad Moradi[d]

[a] Iran National Institute Of Genetic Engineering and Biotechnology, Faculty of Advanced Sciences and Technologies, University of Isfahan, Tehran, Isfahan, Iran
[b] Department of Microbiology, Faculty of Medicine, Kashan University Of Medical Sciences, Kashan, Iran
[c] Department of Microbiology, Faculty of Basic Sciences, Azad University, North Branch, Tehran, Iran
[d] Faculty of Advanced Sciences and Technologies, University of Isfahan, Isfahan, Iran

ARTICLE INFO

Keywords:
pprI gene
Saccharomyces cerevisiae
Deinococcus radiodurans
Biofuel

ABSTRACT

Introduction: Ethanol is considered a comparatively clean biofuel, and its large scale production has been a long time concern. *Saccharomyces cerevisiae* has proven to be the suitable microorganism for large scale ethanol production, but production of other alcohols like butanol and using lignocellulosic substrates is restricted due to lacking tolerance toward toxicity of alcohols, and compounds released from substrates. This study aimed to produce a tolerant strain by using *pprI* gene of *Deinococcus radiodurans*.
Material and method: pprI gene was introduced into Saccharomyces cerevisiae. To evaluate the recombinant gene expression, the qPCR was performed. By Gas chromatography, the yield of ethanol production was measured. To estimate the yield of ethanol production each strain was normally cultured in a treated lignocellulosic substrate. The *S. cerevisiaes* tolerance toward increased salt, ethanol, and butanol concentrations was checked.
Results: Recombinant yeasts tolerated up to 1.2 M salt (7%) and grew well, while normal strain could only survive under 0.85 M (5%) salt concentration. At 5%, 7.5%, 8.5%, 9.5% and 11% ethanol concentrations (v/v), normal cells growth stopped at 7.5% and above; whereas, mutant strains tolerated up to 11% ethanol and proliferated. The mutant yeast's capability to grow in 0.5% and 1% v/v of butanol was raised by 3 and 2.25 fold.
Conclusion: Expression of *pprI* in different cells increases the tolerance toward various compounds including ethanol, salt and butanol along with boosted yield of biofuel production from ligonocellulosic substrate. Mutant strains showed a higher capability of producing alcohol, and cellular tolerance was raised toward growth restricting compounds released from substrates.

1. Introduction

Production of biofuels such as bioalcohols and biodiesels from renewable resources has gained much attention in the past two decades, since energy costs and environmental concerns have raised tremendously. Ethanol, above all, is the most important and widely used biofuel in the world, and more than 50 billion liters of this alcohol is consumed in the USA every year [1].

To date, *Saccharomyces cerevisiae* is considered to be the most acceptable ethanol producing microorganism. Regarding ethanol production, this spherical organism has so many advantages, including its nonpathogenic nature, suitable growth rate, ability to grow on cheap substrates, and capability of bearing high concentrations of ethanol (up to 10% v/v) [2]. Considering these facts, *S. cerevisiae* is an appropriate choice for the production of compounds such as butanol; but there are still major limitations that reduce the efficiency of these processes. Toxicity of Biofuel compounds and inadequate tolerance of S. cerevisiae toward them are among the most important restriction factors.

Low tolerance of cells to different stresses is one of the main challenges in the production of alcohols during bioproduction processes, and increasing the organism's tolerability would certainly escalate the production rate and its efficiency [3]. For instance, it is feasible to boost second-generation biofuel production by raising the ability of cells to tolerate salt and other toxic compounds released from lignocellulosic substrates during treatment. Also, it is necessary to enhance the resistance of the strains when manufacturing butanol. The sensitivity of the cells toward butanol holds back the growth and proliferation rate of the cells and eventually leads to a declined alcohol production [4,5].

Regarding advances in genetic engineering, it is now possible to transfer specific features of various cells to others by expressing the responsible genes. Therefore, by finding the right genes in resistant organisms and transferring them to a suitable strain, it is plausible to

* Corresponding author.
E-mail addresses: Hossein.Helalat@gmail.com, s.hosseinhelalat1397@gmail.com (S. Hossein Helalat).

https://doi.org/10.1016/j.enzmictec.2019.01.008
Received 8 December 2018; Received in revised form 12 January 2019; Accepted 21 January 2019
Available online 23 January 2019
0141-0229/

improve the strain tolerance.

Among microorganisms that tolerate different stress conditions, *Deinococcus radiodurans* is considerably important due to its special properties. This red non-pathogenic gram-positive bacterium forms dyads or tetrads and is the most resistant known microorganism that can survive different environmental stresses including; radiation, extreme dryness, high salt concentration, free radicals, and mitomycin C antibiotic [6]. Its 3.28 megabase pair genome consists of 2 chromosomes and 2 plasmids, and has important genes such as *pprI* which are proved being related to organisms stress tolerance [7].

Analysis Transcriptome of *D. radiodurans* has showed that when this gene is expressed, it produces a metalloprotease enzyme. This enzyme regulates the expression of other genes which are capable to control cellular tolerance. Destruction of the *pprI* gene in *D. radiodurans* causes a drastic decrease in the tolerance of the bacteria to different stresses [8,9]. In addition, transformation and expression of *pprI* in other organisms including plants and bacteria results in distinctive patterns of tolerance [10–13].

Accordingly, due to the high value of biofuel and biobutanol production in *S. cerevisiae*, any approach involving genetic engineering or other methods that could improve functions of this yeast is noteworthy. In the current study heterologous *pprI* gene expression in *S. cerevisiae* was evaluated to measure the enhancement of tolerance toward alcohols and salt, aiming to produce a new stress-tolerant strain in which ethanol production is scaled up.

2. Material and methods

2.1. Strains and culturing conditions

E. coli (DH5α strain) was obtained from The National Institute of Genetic Engineering and Biotechnology of Iran, cultured in LB broth (Trypton 1%, NaCl 1% and 0.5% yeast extract) and incubated at 37 °C employing a shaking incubator (120 rpm).

Uracil negative strain of *S. cerevisiae* CEN.PK 113-5D (At the rest of the article it is called 5D) was obtained from The Faculty of Advanced Science and Technologies of Isfahan University, inoculated in YPD medium (1% yeast extract, 2% peptone and 2% glucose) and was incubated at 30 °C with shaking and aeration (160 rpm).

D. radiodurans was received from Iranian Biological Resource Center (IBRC-M 10,806) and cultivated in TGY medium (0.5% Tryptone, 0.1% Glucose, 0.5% Yeast extract and 0.1% K2HPO4) at the temperature of 30 °C while shaking at 140 rpm. Table 1 contains a list of the utilized and synthesized strains in this study.

2.2. DNA and plasmid extraction

D. radiodurans was cultured in TGY broth under optimum conditions for 24 h; then, 1.5 mL of the bacterial suspension was centrifuged at 9000 *g* for 1 min, the supernatant was discarded and the DNA of residual cells were extracted using DNA Extraction Kit (GENEALL, South Korea). To evaluate the extraction process, the obtained DNA was electrophoresed (agarose 1%).

Table 1
Utilized and synthesized strains in current study.

Microorganism	Characteristics
DH5α	–
CEN.PK 113-5D	Auxotroph, Uracil negative
Scp1	Yeast containing Gppr1 plasmid
Scp2	Yeast containing Gppr2 plasmid
pprmut	Yeast containing Gpprmut plasmid
D. radiodurans	R1 strain
S.c INS	Wild type Yeast strain from national institute of genetic engineering and biotechnology of Iran

Table 2
Plasmids used in current study.

Plasmid Name	Properties
P426GPD	GPD promoter, ura marker, amp marker, 2 μm replication site
GPD-oriless	P426GPD Plasmid lacking replication site 2 μm
Gppr1	P426GPD vector containing pprI gene, lacking replication site 2 μm
Gppr2	P426GPD vector containing pprI gene
Gpprmut	P426GPD vector containing mutated pprI (pprmut) gene

The *E. coli* bacteria harboring plasmid of interest were first cultured in LB broth containing 100 mg/L Ampicillin at 37 °C at 140 rpm for 16 h. To extract the plasmids from *E. coli*, Plasmid extraction kit (GENEALL, South Korea) was used, and the results were evaluated by electrophoresis (agarose 1%). Table 2 enlists the plasmids used in this study.

2.3. P426GPD-oriless Plasmid construction (5382 bp)

In order to construct P426GPD-oriless Plasmid, PWO enzyme (Roche, Swiss) was employed; and plasmids were constructed through PCR method with Foriless and Roriless primers. Primer sequences are listed in Table A.1in supplementary material.

The original plasmid was used as the template in PCR. The primers Foriless and Roriless were used to amplify the fragment lacking the replication site (GPD-oriless) based on the reaction conditions in Table A.2 in supplementary material. The PCR product was then electrophoresed and recovered by a DNA recovery kit (GENEALL, South Korea). At the next step, DNA fragment was digested with Nhel enzyme (Thermo Fisher Scientific, USA) and was again purified. Finally, T4Ligase enzyme (Thermo Fisher Scientific, USA) was used to convert the linear vector to the circular plasmid.

2.4. pprI gene Isolation and cloning

DNA from *D. radiodurans* was extracted and target sequence with the original length of 987 base pairs (in association with primer: 1075 bp), was amplified using Fppr and Rppr primers using Pfu enzyme (Thermo Fisher Scientific, USA) according to the mentioned conditions in Table A.3 in supplementary material.

The PCR product was then electrophoresed in agarose gel to approve the authenticity of the reaction (DNA marker 1Kb, 1.5% agarose gel).

The gene fragments were later recovered from agarose gel, digested with BamHI and EcoRI enzymes (Thermo Fisher Scientific, USA) and purified afterward. P426GPD and GPD-Oriless plasmids were digested with the same enzymes and purified.

For analyzing *pprI* functions in yeast, the metalloprotease region was inactivated by inducing site-directed mutagenesis (*pprmut*). Histidine 86, responsible for attachment of the enzyme to the metal unit; and glutamic acid 83 which is the main amino acid in the active site of the enzyme, were replaced with serine and glutamine respectively [14].

P426GPD and GPD-Oriless were ligated with the prepared insert sequence of *pprI* and *pprmut* genes, exploiting T4Ligase at 16 °C for 16 h.

P426GPD and GPD-Oriless ligation products were separately transformed into the *DH5α* strain of *E.coli*, then cultivated on ampicillin-containing LB Agar plates overnight. On the next day, 5 randomly chosen colonies were inoculated in ampicillin-containing LB broth. Their plasmids were extracted by DNA plasmid extraction kit (Qiagen, Germany). The plasmids were then digested using BamHI and EcoR1 restriction enzymes and checked by agarose gel electrophoresis (agarose 1.5%). Samples which harbored Gppr1 and Gppr2 plasmids were selected for further study.

2.5. Yeast transformation

5D yeast strain was inoculated in YPD broth and incubated for 24-h with shaking (140 rpm) then, lithium chloride transformation method was carried out. Briefly, 24-h old cultures of yeast were grown in a new medium for 5 h and washed with 10 mL LTE buffer (LiCl 0.1 M, Tris—HCl 10 mM, and EDTA 1 mM). Cells were resuspended in 0.5 mL LTE buffer and then allocated into 50 µL microtubes. A microgram of the plasmid and 300 µL of transform buffer (LTE buffer, PEG 3350 [40%]) were relocated to a new microtube and held at 42 °C for 15 min. In the last step, 2 different volumes of the suspension (100 µL and 250 µL) were cultured in minimal Uracil free agar media. Colonies were observed 48 h later.

2.6. Evaluation and confirmation of gene expression in yeast

After 48 h of incubation in YPD, 1.5 mL of the media was centrifuged at 1000 *g* for 5 min. The supernatant was discarded and the RNA was extracted using a RNA extraction kit (Qiagen, Germany). To synthesize the complementary DNA sequence, a cDNA construction kit (Parstus, Iran) was used. PCR was performed with specific primers to confirm the presence of the *pprI* gene.

In order to evaluate the gene of interest expression, the qPCR method was conducted based on the information from Table A.4 in supplementary material using SYBR Green master mix (Parstus, Iran) and *pprI* specific primers. At this step, the tdh3 gene (Housekeeping Gene) was used as the normalizer.

2.7. Ethanol production

To determine each strain's capability to produce ethanol, after 24 h of growing in YPD media, 1% v/v of yeasts were inoculated in 100 mL of YPD media (containing 65 g/L glucose) and incubated at 30 °C with shaking (120 rpm). Two samples were taken 24 and 72 h later. The total volume of glucose in the media was checked after 72 h. When it was fully consumed, glucose was freshly added gradually until making it 300 g/L at the end. On the 7th day, sample was taken from the media again for GC analysis.

Furthermore, each strain was normally cultured at 30 °C with shaking (120 rpm) in a treated lignocellulosic substrates in order to estimate the amount of ethanol production in this substrates, then samples were taken after 48 h.

To treat the lignocellulosic substrate, 1 g of wheat bran and straw was added to 40 mL distilled water with a pH of 2, and then, the combination was autoclaved. Afterward, the pH was neutralized. At the next step, xylanase and cellulase enzymes from camel rumen Metagenome [15] were added and the mixture was held at room temperature for 16 h and then filtered using a filter paper (0.45 µm). The obtained liquid was used as a medium for yeasts culturing. All steps were repeated 3 times to ensure the reliability of the results.

2.8. Measuring ethanol production in yeast strains

Gas chromatography was performed to measure the yield of ethanol production. Varian CP-3800 device was used in the following conditions: CP-Wax 57 column with the length of 25 m and diameter of 0.32 mm, film thickness of 1.2 µm, FID detector, and 12 psi column pressure.

2.9. Sample preparation

To make the samples ready for injection to the column, 1.2 mL of yeast-containing medium was centrifuged at 1500 *g* for 5 min to separate the cells from the supernatant. The medium was then passed through a 0.22 µm filter. The filtered medium, plus 30 mg of methanol as an internal control, were brought to the volume of one mL; and one µL of this mixture was injected to the device.

To quantify the produced ethanol, samples containing specific concentrations of ethanol (5, 10, 25 and 100 mg/mL) and internal control (methanol 30 mg/mL) were injected to the column and the resulted standard graphs were obtained and used to quantify the unknown measures.

2.10. Assessment of pprI gene expression on cellular growth and stress tolerance

Following the *pprI* gene expression, and prior to analyzing alterations in strains, the effect of *pprI* expression on growth was examined under normal culture conditions. To evaluate its effect, 5D, Scp1, and Scp2 strains were inoculated in YPD media. The optical density (OD) of each sample was measured at 650 nm every 12 h. This procedure was repeated 3 times.

The tolerance of Scp1, Scp2, Pprmut and 5D strains to increased concentrations of salt, ethanol, and butanol was checked by culturing them in Different concentrations of added salt (%2, % 3, %4, %5 and %7), ethanol (%5, %7.5, %8.5, %9.5 and %11 v/v) and butanol (%0.5, %1, %1.25, %1.5 and %2 v/v) in YPD media and then OD (650 nm) was evaluated for each sample after 48 h.

3. Results

3.1. Real-time PCR result assessment

According to delta-delta CT analysis, the expression rate of the *pprI* gene was at least 30 times lower when Gppr1 vector was used, compared to that of Gppr2 vector. It could be due to the fact that a replication origin does not exist in the vector, thus it enters the yeast's genome.

3.2. Studying pprI gene function on yeast growth

According to the comparison of the optical density analysis (OD) results from normal yeast (5D) and recombinant strains (Scp1 and Scp2) presented in Fig. 1 diagram, under the normal growth condition, the presence of the *pprI* gene in the cell does not appear to have any considerable impact on cellular growth rate, although, at some growth stage in Scp2, it seems that the recombinant strain accelerated the rate, but final stage appears not to be affected.

3.3. Influence of pprI gene on stress tolerance in S. cerevisiae

The tolerance of *S. cerevisiae* to increased concentrations of salt, ethanol, and butanol was checked. Results are shown in Fig. 2–4. No

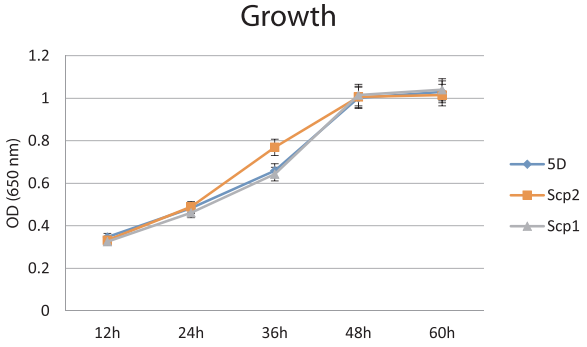

Fig. 1. Evaluating growth rate of Scp1 and Scp2 mutant strains in comparison with normal 5D strain in YPD media. Culturing was performed at 30 °C with 160 rpm shaking in identical normal culturing condition. Cellular growth was measured by OD analysis (650 nm) each 12 h until 60 h. Error bars indicate standard deviation at three times repeats for each sample.

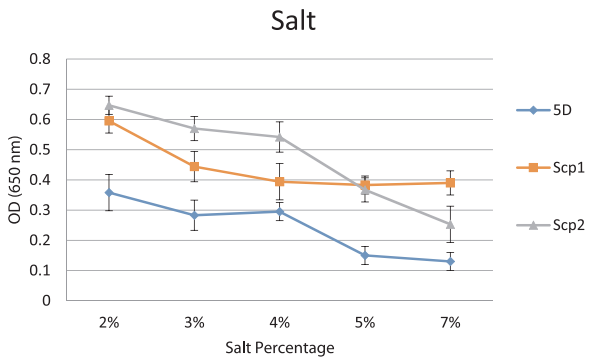

Fig. 2. Comparative evaluation of salt tolerance among Scp1 and Scp2 mutant strains, and normal 5D strain. Culturing at YPD media was performed at 30 °C with 160 rpm shaking in identical condition to indicate their tolerance toward salt at %2, %3, %4, % 5, and %7 concentrations. After 48 h culturing, OD analysis (650 nm) was carried out. Error bars indicate standard deviation at three times repeats for each sample.

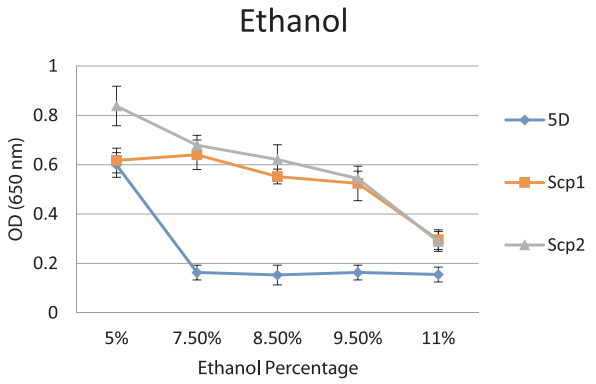

Fig. 3. Comparative evaluation of ethanol tolerance among Scp1 and Scp2 mutant strains, and normal 5D strain. Culturing at YPD media was performed at 30 °C with 160 rpm shaking in identical condition to indicate their tolerance toward ethanol at %5, %7.5, %8.5, % 9.5 and %11 concentrations. After 48 h culturing, OD analysis (650 nm) was carried out. Error bars indicate standard deviation at three times repeats for each sample.

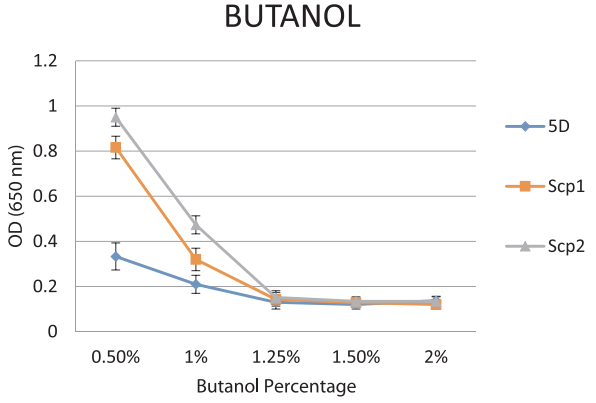

Fig. 4. Comparative evaluation of ethanol tolerance among Scp1 and Scp2 mutant strains, and normal 5D strain. Culturing at YPD media was performed at 30 °C with 160 rpm shaking in identical condition to indicate their tolerance toward butanol at %0.5, %1, %1.25, %1.5 and %2 concentrations. After Culturing for 48 h OD analysis at 650 nm was carried out. Error bars indicate standard deviation at three times repeats for each sample.

changes in the tolerance of pprmut strain containing the mutant gene were observed. This is the first evidence confirming that this gene has a metalloprotease function in yeasts.

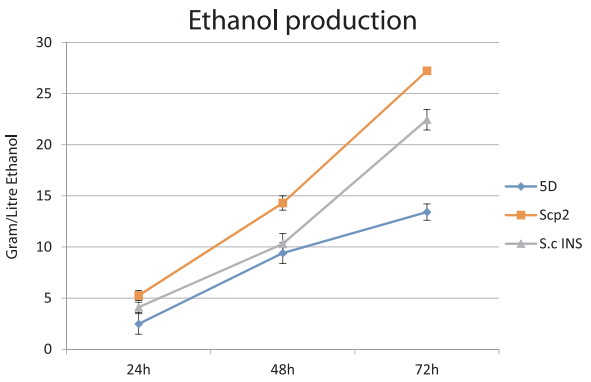

Fig. 5. Evaluating the amount of ethanol production (g/L) by mutant strain (Scp2), normal strain (5D) and wild type (S.C INS). Culturing at YPD media was performed at 30 °C with 160 rpm shaking in identical condition for 24, 48, and 72 h. The amount of ethanol production was measured by gas chromatography (GC). The figure has been depicted according to the standard diagram of GC analysis. Error bars indicate standard deviation at three times repeats for each sample.

According to results of OD analysis, it was indicated that in the same culturing condition, yeasts harboring the recombinant *pprI* gene, tolerated up to 1.2 M salt (7%) and grew in an acceptable manner, while normal strain could only survive in salt concentrations lower than 0.85 M (5%). Comparative results of OD analysis for this step is presented in Fig. 2.

OD analysis performed at 5%, 7.5%, 8.5%, 9.5% and 11% ethanol concentrations (v/v) indicated that normal cells growth stopped at 7.5% ethanol concentrations and above that; whereas, Scp1 and Scp2 strains were still able to tolerate up to 11% ethanol and grow in the YPD media (Fig. 3).

By the similar OD analysis, it was shown that The yeast's capability to grow in 0.5% and 1% v/v of butanol was raised up to 3 and 2.25 folds (Fig. 4).

3.4. Impact of pprI gene on alcohol production

The Scp2 mutant strain produced 27.23 ± 1.24 g of ethanol per Liter in 72 h, while the normal strain made only 13.41 ± 1.83 g/L (Fig. 5). This clearly indicates that the ability to produce ethanol was increased by 2 fold when this gene was present. On the other hand, Scp2 strain had worked more efficiently compared to wild type strain S.c INS and produced more ethanol. The total volume of glucose in the media was checked after 72 h. After adding glucose to 300 g/L, On the 7th day The highest yield of ethanol, equal to 123.5 ± 1.52 g/L, belonged to Scp2 strain. The normal and the wild strains produced 113.6 ± 1.67 g/l and 117.5 ± 1.87 g/l, respectively (Fig. 6).

In the next step, all 4 yeast strains were inoculated in treated

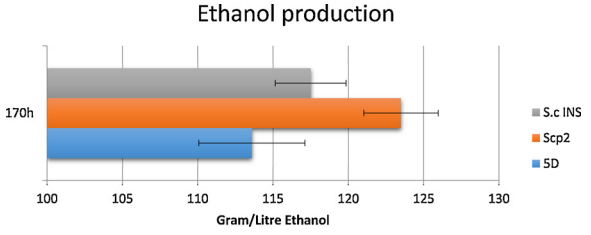

Fig. 6. Evaluating the amount of ethanol production (g/L) by mutant strain (Scp2), normal strain (5D) and wild type (S.C INS) in YPD culturing media at 30 °C with 160 rpm shaking by gradual addition of glucose up to 300 g/L during 170 h fermentation. The quantitative measurement of ethanol production was performed by GC analysis. Error bars show standard deviation at three times repeats for each sample. (To understand the references to color in this figure legend, the reader is referred to the web version of this article.).

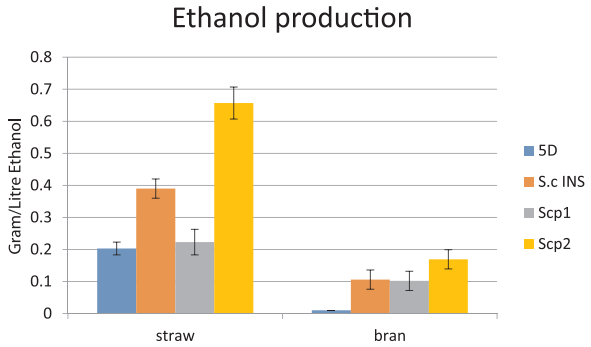

Fig. 7. Evaluating the amount of ethanol production (g/L) by mutant strains (Scp1 and Scp2), normal strain (5D) and wild type (S.C INS) on the lignocellulosic substrate. Substrates were treated with acidic pH, and high temperature. Subsequent to neutralization, xylanase, and cellulase enzymes were added to the media. The fermentation process was performed for 48 h at 30 °C with 160 rpm shaking. Error bars show standard deviation at three times repeats for each sample. (To understand the references to color in this figure legend, the reader is referred to the web version of this article.).

lignocellulosic media. Samples were taken and analyzed for ethanol production. Results indicated that tolerant strains could produce more ethanol, as is the case for Scp2 and Scp1 strains which made 0.657 ± 0.051 g/L and 0.223 ± 0.049 g/L in straw medium, respectively, while the normal strain (5D) produced 0.203 ± 0.019 g/L of ethanol and the wild type (S.C.INS) secreted 0.39 ± 0.038 g/L. In treated bran media, Scp1 and Scp2 strains were able to make 0.169 ± 42 g/L and 0.102 ± 0.043 g/L respectively, whereas, the wild strain could produce 0.106 ± 0.041 g/L and normal cells could merely produce 0.01 ± 0.002 g/L of ethanol (Fig. 7).

4. Discussion

PPRI protein is a global regulator (also known as IrrE) that has shown to support cellular resistance in *Deinococcaceae*. The IrrE protein has mostly been studied for its regulatory role in resistance of *D. radiodurans* against environmental stresses [16–18]. It has been suggested earlier that the protease activity of this protein could be important in transcription of target genes [14]. Previous studies of heterologous expression of *IrrE* in bacteria have indicated that it increases the stress tolerability of the recombinant strains [19,20]. A study of evaluating the role of *IrrE* in *E.coli* has proposed that IrrE protein imposes its function by stimulating transcription of *recA* gene upon radiation exposure [21].

Previously the effect of heterologous *IrrE* gene expression in Ethanologenic *E.coli* has been evaluated, and it has been proposed that it could improve osmotic tolerance of the bacterial cells along with higher production of ethanol by the bacteria [22]. In a similar study done by Jie Pan et al. in 2009, the role of the *pprI* gene in *E. coli* and also a plant named *Brassica napus* was determined. Their results indicated that when this gene was expressed, it assisted the organism to tolerate 350 mM salt and grow perfectly after 6 weeks, while the plant lacking this gene could not survive in this condition. *E. coli* strains which had the gene, when placed in a medium containing 3 M salt for 2 h, were more resistant comparing to the ones that lacked *pprI* gene. In addition, the bacteria possessing the gene were able to reach their maximum growth in 0.65 M salt concentration after 60 h of incubation under optimum conditions, but the normal cells had no growth at all [10].

In the same research, conducting Real-Time PCR, more data was obtained from transcriptome analysis of *B. napus*, clearly showed that the expression of *pprI* in this organism alters the expression rate of other genes involved in stress response [8].

In another study, Zhang et al. expressed the *pprI* gene in *Zymomonas mobilis* and studied the role of this gene in gaining tolerance to acids and ethanol. During this study it was revealed that the *pprI* gene amplifies the expression and function of pyruvate decarboxylase and alcohol dehydrogenase; as consequence, the bacteria were more tolerant to lower levels of pH and higher amounts of ethanol. In the same study, the bacteria which had the *pprI* gene compared to the wild strain had better growth in the media containing 12% ethanol during 60 h. The results showed that the mutant *Z. mobilis*, was more tolerant compared to the wild strain [13]. The provided information from this research elucidated that this gene has a similar tolerability effect in *Z.mobilis*.

In the current study, the effects of the *pprI* gene on ethanol production of *S. cerevisiae* and its tolerance to salt, ethanol, and butanol was analyzed. The results showed samples which had the *pprI* gene were more tolerant. To evaluate the osmotic fragility of the cells, and assess the resistance toward salt, various concentrations of NaCl were applied and the cell's growth rate was monitored in a period of 48 h.

Based on the findings from current research and previous studies, it can be stated that the expression of *pprI* gene in different cells intensifies the organism's tolerance to salt. OD analysis results of yeast growth in YPD media with extra salt demonstrated that *pprI* recombinant strains may resist up to 7% salt (1.2 M) and grew normally, while normal strain without the gene of interest only survived in under 5% (0.85 M) salt concentration.

In regards to the fact that the most important function of *S. cerevisiae* is in industrial ethanol production, and noting the worldwide significance of ethanol production, we tracked the changes in tolerance levels of the cells at various ethanol concentrations. According to the results obtained from the tests performed for 5%, 7.5%, 8.5%, 9.5% and 11% ethanol concentrations (v/v), normal cells growth stopped at 7.5% and above; whereas, Scp1 and Scp2strains could tolerate up to 11% ethanol and grow, proving the higher ethanol resistance characteristics in recombinant strains.

In the current study, we also determined ethanol production potency in modified *S. cerevisiae* by gas chromatography, and it was revealed that yeast strains which were more tolerant, could produce higher amounts of ethanol in comparison to normal strains. To monitor the enhancements in the functions of the engineered strains, the yield of ethanol was compared with the wild strain. The production rate of engineered strains was almost two times higher than the normal strain under laboratory conditions, reflecting the exceptional properties of *pprI* gene in yeast. Also, ethanol yield was 3 times higher when yeasts were grown in a treated lignoocellulosic medium. These findings could be helpful in designing more efficient *S. cerevisiae* strains.

In the present study, in addition to changes in levels of resistance to ethanol, we also studied perseverance of synthesized strains while growing in the presence of butanol. Since butanol, as a clean biofuel, is known to be superior to ethanol, much effort has been made to modify the strains which produce this substance. Low tolerance of these strains including *S.cerevisiae*, has always been a major problem in butanol production. Thus, any measure improving the strain's tolerance might escalate the production [23]. Results show the yeast's capability to grow in 0.5% and 1% v/v of butanol was raised by 3 and 2.25 folds in recombinant strains, which implies that the tolerance was elevated in a considerable manner when compared to wild strain.

We also determined ethanol production potency in modified *S. cerevisiae* by gas chromatography, and it was revealed that yeast strains which were more tolerant, could produce higher amounts of ethanol in comparison to normal strains. To monitor the enhancements in the functions of the engineered strains, the yield of ethanol was compared with a wild strain. The production rate of engineered strains was almost two times higher than the normal strain under laboratory conditions, reflecting the exceptional properties of *pprI* gene in yeast.

Finally, it is worthy of mention that results of gas chromatography assay demonstrated that the strains harboring this particular gene, have a higher capability of producing ethanol in treated lignocellulosic media, since this gene may enhance cellular tolerance to restricting effects of the compounds released from lignocellulosic materials;

therefore, influences ethanol production. These findings could be helpful in designing more efficient *S. cerevisiae* strains.

5. Conclusion

It was observed that expression of *pprI* gene in yeast cells brings about changes in tolerablity to ward a wide range of compounds including ethanol, and butanol along with increased yield of biofuel production from lignocellulosic substrates. This modification can have a major influence on butanol production in engineered *S. cerevisiae* strains.

Declarations of interest

None.

Authors contribution

S. Hossein Helalat: conceptualization of Idea, research conduct, supervision, experiment
Shirin Bidaj: experimental research
Sadaf Samani: experimental research
Mohammad Moradi: experimental research

Acknowledgement

We would like to thank Dr Mohammad Ali Asadollahi of Isfahan university for his kind contribution to this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.enzmictec.2019.01.008.

References

[1] J. Hill, E. Nelson, D. Tilman, S. Polasky, D. Tiffany, Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels, Proc. Natl. Acad. Sci. U S A 103 (30) (2006) 11206–11210.
[2] N.A. Buijs, V. Siewers, J. Nielsen, Advanced biofuel production by the yeast Saccharomyces cerevisiae, Curr. Opin. Chem. Biol. 17 (3) (2013) 480–8488.
[3] F.H. Lam, A. Ghaderi, G.R. Fink, G. Stephanopoulos, Biofuels. Engineering alcohol tolerance in yeast, Science. 346 (6205) (2014) 71–75.
[4] E.J. Steen, R. Chan, N. Prasad, S. Myers, C.J. Petzold, A. Redding, et al., Metabolic engineering of Saccharomyces cerevisiae for the production of n-butanol, Microb. Cell Fact. 7 (2008) 36.
[5] S.S. Vinay Sharma, Arindam Kuila, A review on current technological advancement of lignocellulosic bioethanol production, J. Appl. Biotechnol. Bioeng. 1 (2) (2016) 61–66.
[6] D. Slade, M. Radman, Oxidative stress resistance in Deinococcus radiodurans, Microbiol. Mol. Biol. Rev. 75 (1) (2011) 133–191.
[7] K.S. Makarova, L. Aravind, Y.I. Wolf, R.L. Tatusov, K.W. Minton, E.V. Koonin, et al., Genome of the extremely radiation-resistant bacterium Deinococcus radiodurans viewed from the perspective of comparative genomics, Microbiol. Mol. Biol. Rev. 65 (1) (2001) 44–79.
[8] H. Lu, G. Gao, G. Xu, L. Fan, L. Yin, B. Shen, et al., Deinococcus radiodurans PprI switches on DNA damage response and cellular survival networks after radiation damage, Mol. Cell Proteomics 8 (3) (2009) 481–494.
[9] A.M. Earl, M.M. Mohundro, I.S. Mian, J.R. Battista, The IrrE protein of Deinococcus radiodurans R1 is a novel regulator of recA expression, J. Bacteriol. 184 (22) (2002) 6216–6224.
[10] J. Pan, J. Wang, Z. Zhou, Y. Yan, W. Zhang, W. Lu, et al., IrrE, a global regulator of extreme radiation resistance in Deinococcus radiodurans, enhances salt tolerance in Escherichia coli and Brassica napus, PLoS One 4 (2) (2009) e4422.
[11] X. Dong, B. Tian, S. Dai, T. Li, L. Guo, Z. Tan, et al., Expression of PprI from Deinococcus radiodurans improves lactic acid production and stress tolerance in Lactococcus lactis, PLoS One 10 (11) (2015) e0142918.
[12] R. Ma, Y. Zhang, H. Hong, W. Lu, M. Lin, M. Chen, et al., Improved osmotic tolerance and ethanol production of ethanologenic Escherichia coli by IrrE, a global regulator of radiation-resistance of Deinococcus radiodurans, Curr. Microbiol. 62 (2) (2011) 659–664.
[13] Y. Zhang, R. Ma, Z. Zhao, Z. Zhou, W. Lu, W. Zhang, et al., irrE, an exogenous gene from Deinococcus radiodurans, improves the growth of and ethanol production by a Zymomonas mobilis strain under ethanol and acid stress, J. Microbiol. Biotechnol. 20 (7) (2010) 1156–1162.
[14] A. Vujičić-Žagar, R. Dulermo, M. Le Gorrec, F. Vannier, P. Servant, S. Sommer, et al., Crystal structure of the IrrE protein, a central regulator of DNA damage repair in Deinococcaceae, J. Mol. Biol. 386 (3) (2009) 704–716.
[15] K. Khalili Ghadikolaei, K. Akbari Noghabi, H. Shahbani Zahiri, Development of a bifunctional xylanase-cellulase chimera with enhanced activity on rice and barley straws using a modular xylanase and an endoglucanase procured from camel rumen metagenome, Appl. Microbiol. Biotechnol. 101 (18) (2017) 6929–6939.
[16] Y. Hua, I. Narumi, G. Gao, B. Tian, K. Satoh, S. Kitayama, et al., PprI: a general switch responsible for extreme radioresistance of Deinococcus radiodurans, Biochem. Biophys. Res. Commun. 306 (2) (2003) 354–360.
[17] B. Joshi, R. Schmid, K. Altendorf, S.K. Apte, Protein recycling is a major component of post-irradiation recovery in Deinococcus radiodurans strain R1, Biochem. Biophys. Res. Commun. 320 (4) (2004) 1112–1117.
[18] A. Bauermeister, E. Bentchikou, R. Moeller, P. Rettberg, Roles of PprA, IrrE, and RecA in the resistance of Deinococcus radiodurans to germicidal and environmentally relevant UV radiation, Arch. Microbiol. 191 (12) (2009) 913.
[19] T. Chen, J. Wang, L. Zeng, R. Li, J. Li, Y. Chen, et al., Significant rewiring of the transcriptome and proteome of an Escherichia coli strain harboring a tailored exogenous global regulator IrrE, PLoS One 7 (7) (2012) e37126.
[20] G. Gao, B. Tian, L. Liu, D. Sheng, B. Shen, Y. Hua, Expression of Deinococcus radiodurans PprI enhances the radioresistance of Escherichia coli, DNA Repair (Amst.) 2 (12) (2003) 1419–1427.
[21] A.M. Earl, M.M. Mohundro, I.S. Mian, J.R. Battista, The IrrE protein of Deinococcus radiodurans R1 is a novel regulator of recA expression, J. Bacteriol. 184 (22) (2002) 6216–6224.
[22] R. Ma, Y. Zhang, H. Hong, W. Lu, M. Lin, M. Chen, et al., Improved osmotic tolerance and ethanol production of ethanologenic Escherichia coli by IrrE, a global regulator of radiation-resistance of Deinococcus radiodurans, Curr. Microbiol. 62 (2) (2011) 659–664.
[23] T. Lutke-Eversloh, H. Bahl, Metabolic engineering of Clostridium acetobutylicum: recent advances to improve butanol production, Curr. Opin. Biotechnol. 22 (5) (2011) 634–647.